

PIV mutants having ts, ca or att phenotypes, are incorporated within recombinant PIV clones. These changes from biologically derived mutant PIV specify desired characteristics in the resultant clones, e.g., an attenuation phenotype specified by a mutation adopted from the HPIV3 mutant JS cp45 deposited in accordance with the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Blvd. Manassas, VA 20110-2209, U.S.A., and granted the designation PTA-2419. These changes are preferably introduced into recombinant virus using two or three nucleotide changes compared to a corresponding wild type or biologically derived mutant sequence, which has the effect of stabilizing the mutation against genetic reversion. *W*

IN THE CLAIMS:

Please amend the claims as follows:

RECEIVED

APR 12 2001

TECH CENTER 1600/2900

11. (Twice Amended) An isolated polynucleotide molecule comprising an operably linked transcriptional promoter, a polynucleotide sequence encoding a human or bovine PIV genome or antigenome, and a transcriptional terminator, wherein said polynucleotide sequence encoding said PIV genome or antigenome is modified by a nucleotide insertion, rearrangement, deletion or substitution, whereby said polynucleotide upon coexpression with PIV N, P and L proteins yields an infectious PIV particle.

39. (Twice Amended) The isolated polynucleotide molecule of claim 33, wherein said chimeric genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present in rcp45, rcp45 3'NCMFHN, rcp45 3'NL, rcp45 3'N, or rcp45 F other than mutations in HN and F, selected from i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

55. (Amended) The method of claim 52, wherein the N, P and L proteins are encoded on three different expression vectors.

131. (Amended) The isolated polynucleotide molecule of claim 130, wherein said one or more mutations of JS cp45 comprise a plurality and up to a full complement of mutations present in JS cp45 other than mutations in HN and F, selected from i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

132. (Three Times Amended) The isolated polynucleotide molecule of claim 129, wherein the isolated polynucleotide encoding the chimeric PIV genome or antigenome further incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

134. (Three Times Amended) The isolated polynucleotide molecule of claim 133, wherein said chimeric genome or antigenome incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in

6 the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position
7 corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at
8 nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene
9 start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS
10 cp45.

1 136. (Three Times Amended) The method of claim 135, wherein said
2 genome or antigenome incorporates mutations comprising i) substitutions specifying a
3 replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L
4 protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N
5 protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv)
6 mutations in a 3' leader sequence comprising a T to C change at a position corresponding to
7 nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28,
8 and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence
9 comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

1 139. (Amended) The isolated infectious PIV particle of claim 138, wherein
2 said one or more mutations of JS cp45 comprise a plurality and up to a full complement of
3 mutations present in JS cp45 other than mutations in HN and F, selected from i) substitutions
4 specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the
5 polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for
6 Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C
7 protein iv) mutations in a 3' leader sequence comprising a T to C change at a position
8 corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at
9 nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene
10 start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS
11 cp45.

1 140. (Three Times Amended) The isolated infectious PIV particle of claim
2 137, wherein the isolated polynucleotide encoding the chimeric PIV genome or antigenome
3 further incorporates mutations comprising i) substitutions specifying a replacement of His for

4 Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions
5 specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a
6 substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3'
7 leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS
8 cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change
9 at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to
10 T change at a position corresponding to nucleotide 62 of JS cp45.

141. (Three Times Amended) The isolated infectious PIV particle of claim
111, wherein said chimeric PIV genome or antigenome further incorporates mutations
comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile
for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for
Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr
for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a
position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T
change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in
an N gene start sequence comprising an A to T change at a position corresponding to
nucleotide 62 of JS cp45.

143. (Three Times Amended) The immunogenic composition of claim 142,
wherein said recombinant PIV genome or antigenome further incorporates mutations
comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile
for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for
Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr
for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a
position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T
change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in
an N gene start sequence comprising an A to T change at a position corresponding to
nucleotide 62 of JS cp45.